

1/18

Stability of HA-hGH at 37°C in cell culture media

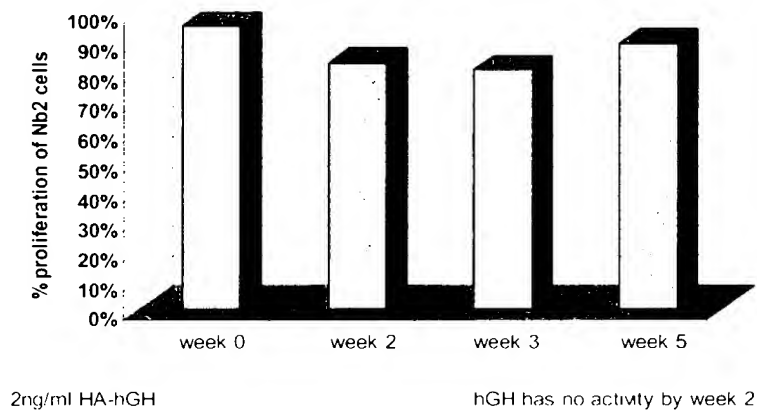
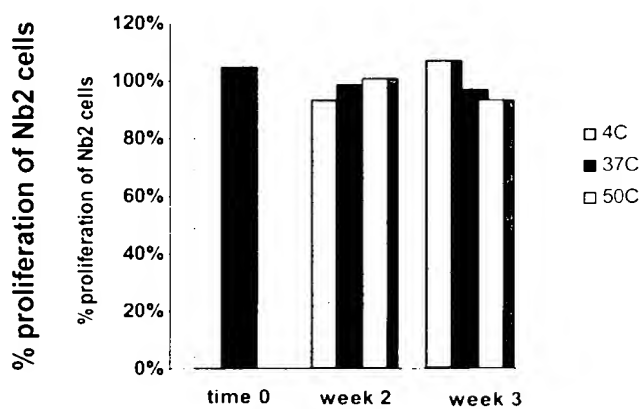


Figure 1

2/18

Stability of HA-hGH in cell culture media



60ng/ml of HA-hGH

Figure 2

3/18

Nb2 Cell Proliferation Assay (24hrs)

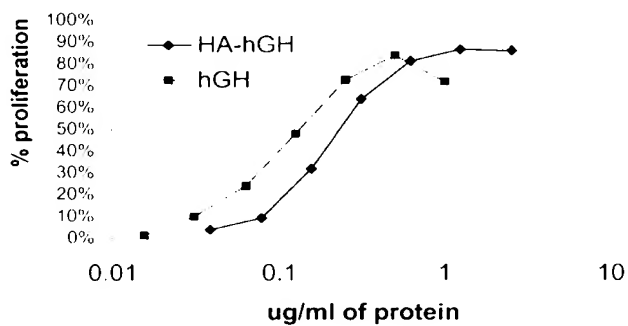


Figure 3A

Nb2 Cell Proliferation Assay (48hrs)

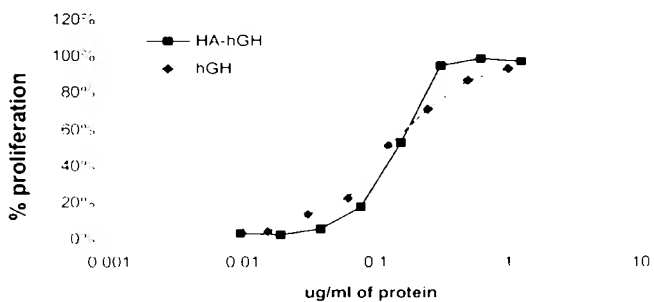


Figure 3B

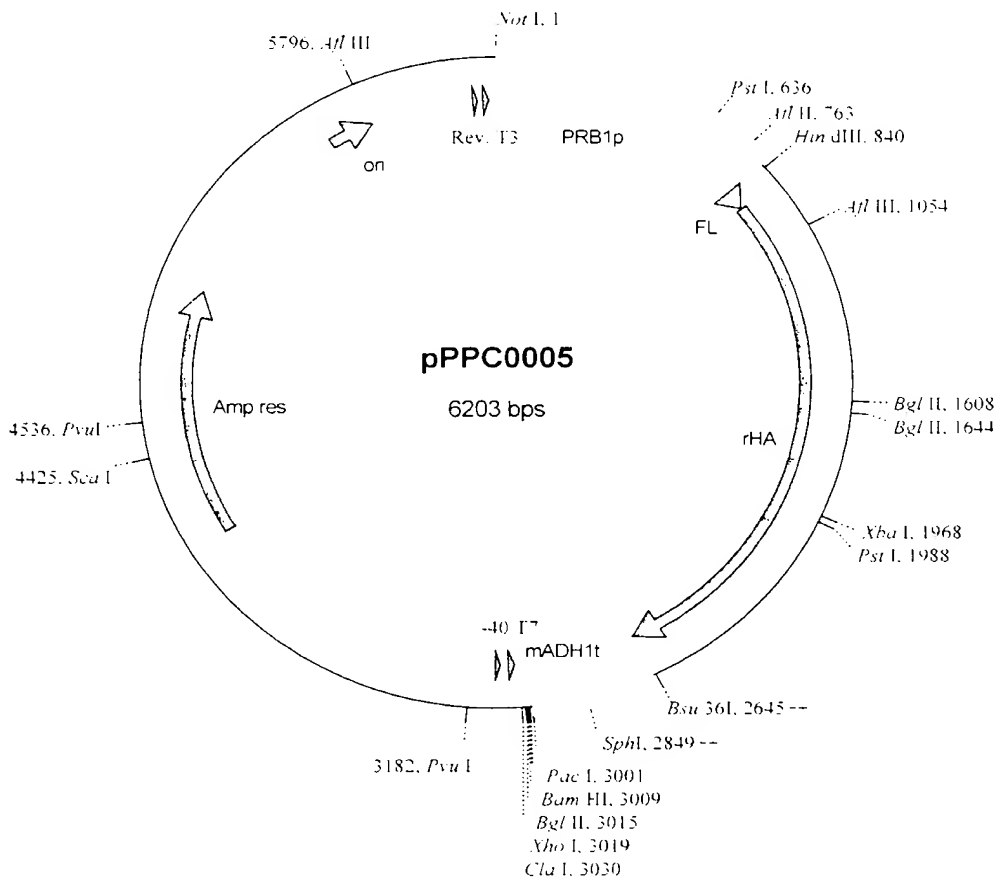


Figure 4

5/18

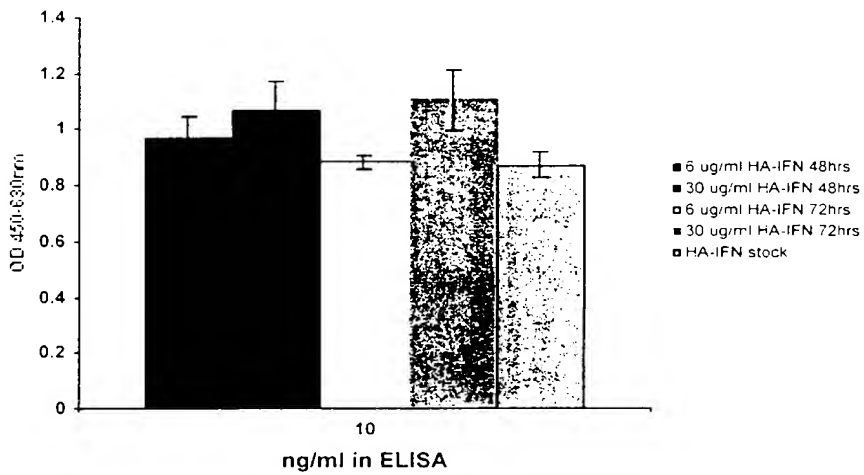


Figure 5

Figure 6

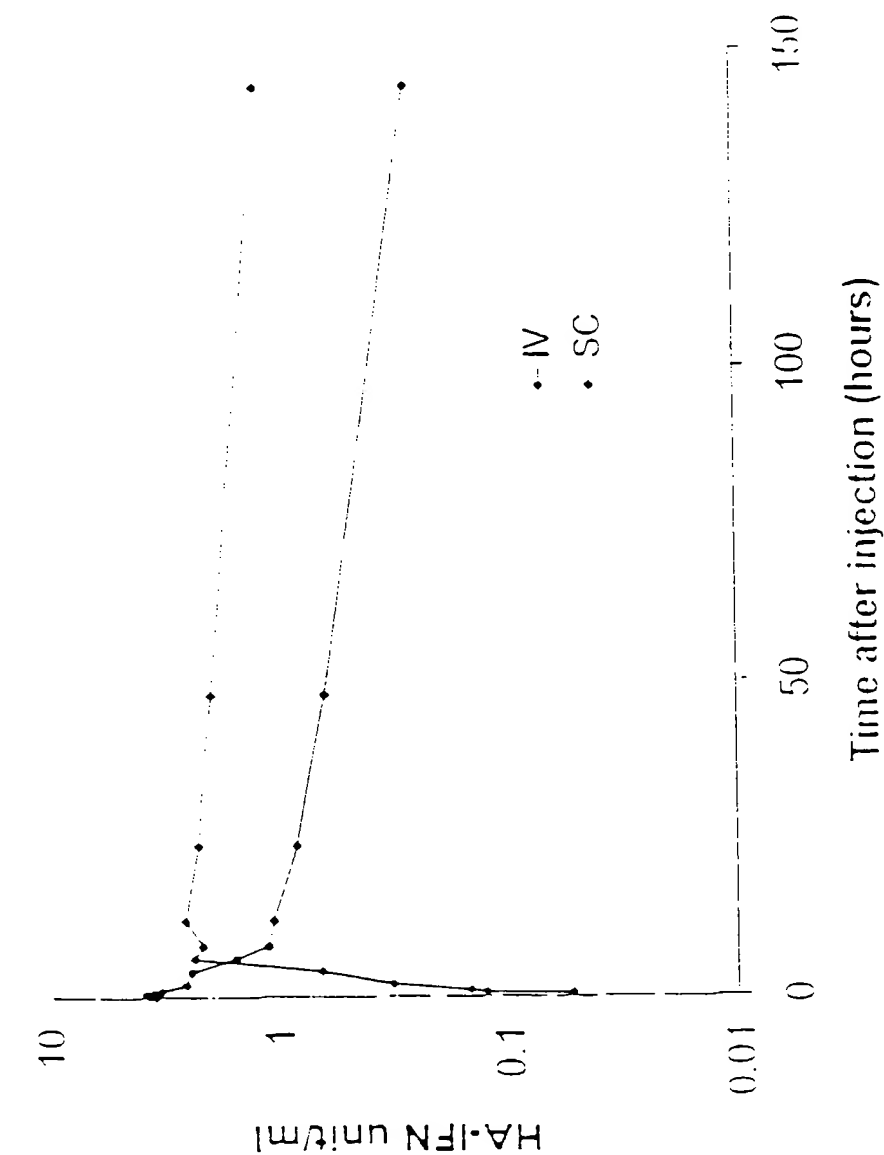
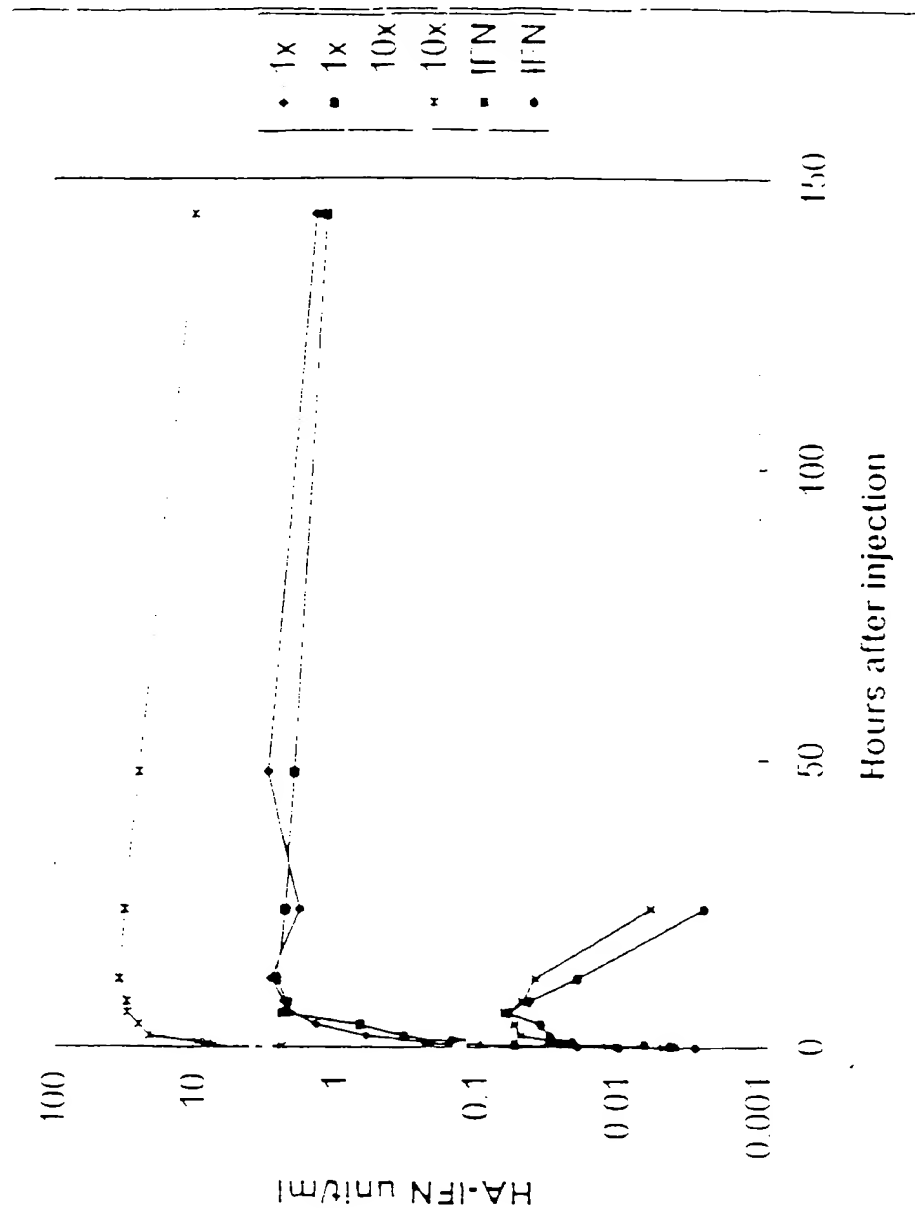


Figure 7



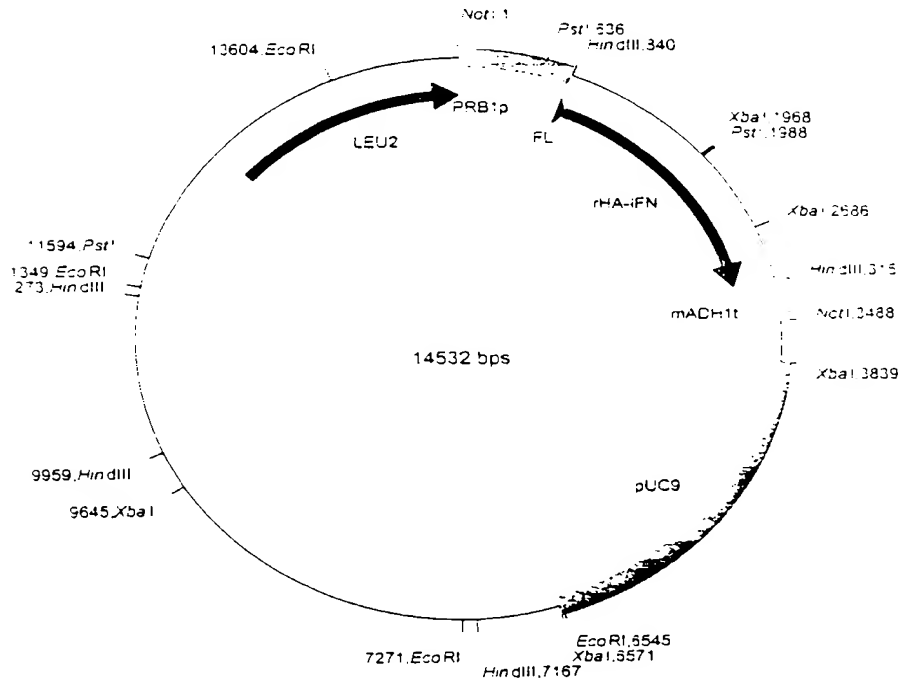


Figure 8. The HA-IFN α expression cassette in pSAC35. The expression cassette comprises
PRB1 promoter, from *S. cerevisiae*.
 Fusion leader, first 19 amino acids of the HA leader followed by the last 6 amino acids of the MF α -1 leader.
 HA-IFN α coding sequence with a double stop codon (TAATAA)
ADH1 terminator, from *S. cerevisiae*. Modified to remove all the coding sequence normally present in the *Hind* III/*Bam* HI fragment generally used.

Figure 8

9/18

Localisation of 'Loops' based on the HA Crystal Structure
which could be used for Mutation/Insertion

```

1    DAHKSEVAHR FKLOGEENFK ALVLIAPAQY LQQCPFEDHV KLVNEVTEFA
      HHHHH HHH HHH HHHHHHHHHH HHHHH HHHHHHHHHH

51   I          II          III
    KCTVADESAE NCDKSLHTLF GDKLCTVATL RETYGEMADG CAKOEPERNE
      HHHHH HHHHH HHHHH HHHH H HHHH

101  CFLQHKDDNP NLPRLVRPETV DVMCTAFMDN SETFLKKYLY EIARRHPYFY
      HHHH H HHHHHHHH HHHHHHHHH HHHHH

151  IV
    APDLLFFAKR YKAAPTECCO AADKAACLLP KLDLDRDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHH HHHHH HHHHHHHHHH HHHHHHHHHH

201  V
    ASLQKFGERA FKAWAVARLS QRFPKAEFAE VSKLVTDLTG VHTECCHGDL
      HHHHH HH HHHHHHHHHH HH HHH HHHHHHHHHH HHHHHH HH

251  VI          VII
    LECADDAEAL AKYICENODS ISSKLKECCE KPLLEKSHCI AEVENDEMFA
      HHHHHHHHHH HHHHH HHHHH HHHHHHH H

301  DLPSLAADFV ESKDVCKNYA EAKDVFLCMF LYEVARRHPD YSVVLLRLA
      HHHH HHHHHH HHHHHHH HHHHH HHHHHHHH

351  VIII
    KTYETTLKCK CAAADPHECY AKVFDEPKPL VEEPQNLIKQ NCLEFEQLGE
      HHHHHHHHHH HH H HHHHH HHHHHHHHHH HHHHHH

401  IX
    YKFNALLVR YTKKVPQVST PTLVEVSENL GKVGSKCKKH PEAKRMPCAE
      HHHHHHHHHH HHHH H HHHHHHHHH HHH HHHHHHHH

451  X          XI
    DYLSVVLNQL CVLHEKTPVG DRVTKCTES LVNRRPPTGSA LEVDETYVPK
      HHHHHHHHHH HHHHH HHHHHHHHH HHHHHHHH

501  EFNAETTFTH ADICTLSEKE RQIKKQTAIV ELVKKHSPAT KEQLFAYMDD
      HHH HHH HHHHHHHHHH HHH HHHHHHHHHH

551  XII
    FAAFVEXCKK ADDKETCFAE EGKALVAASQ AALGL
      HHHHHHHH HHHH HHHHHHHHHH HH
  
```

Loop		Loop	
I	Val154-Asn161	VII	Glu280-His283
II	Thr176-Asp183	VIII	Ala343-Glu349
III	Ala392-Glu400	IX	Lys439-Pro447
IV	Gln172-Ala174	X	Val442-Lys475
V	His247-Glu252	XI	Thr473-Pro496
VI	Glu268-Glu277	XII	Lys580-Thr596

Figure 9

10/18

Examples of Modifications to Loop IV

a. Randomisation of Loop IV.

```
IV
151  APPELLFFAKR YKAAPTCCQ AADKAACLLP KLDELRLDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHHH      HHHHH HHHHHHHHHHH HHHHHHHHHH
```

```
IV
151  APPELLFFAKR YKAAPTCCX XXXXXXCLLP KLDELRLDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHHH      HHHHH HHHHHHHHHHH HHHHHHHHHH
```

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

b. Insertion (or replacement) of Randomised sequence into Loop IV.

'X'.



IV

```
151  APPELLFFAKR YKAAPTCCQ AADKAACLLP KLDELRLDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHHH      HHHHH HHHHHHHHHHH HHHHHHHHHH
```

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 28.

Figure 10

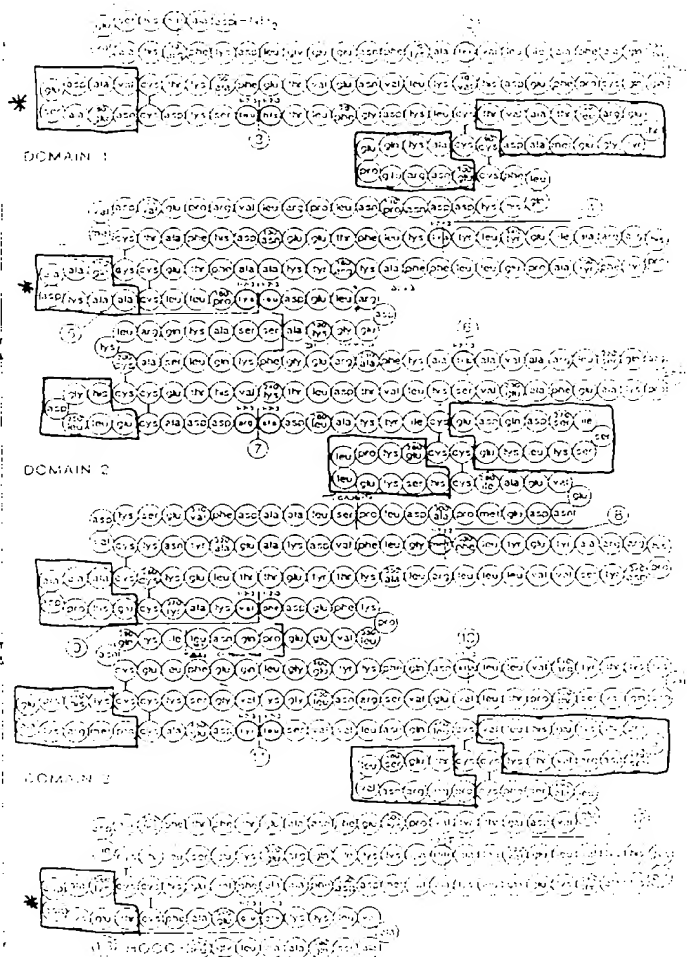
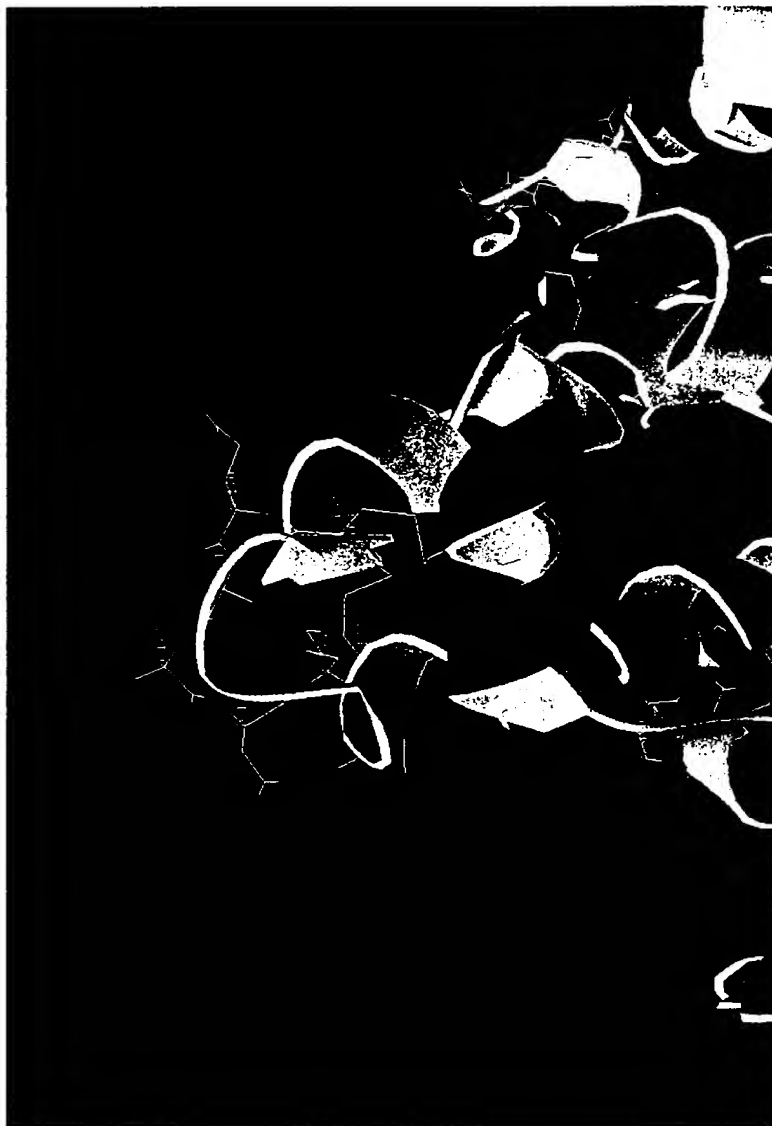


Figure 11



Disulfide bonds shown in yellow

Figure 12: Loop IV Gln170-Ala176

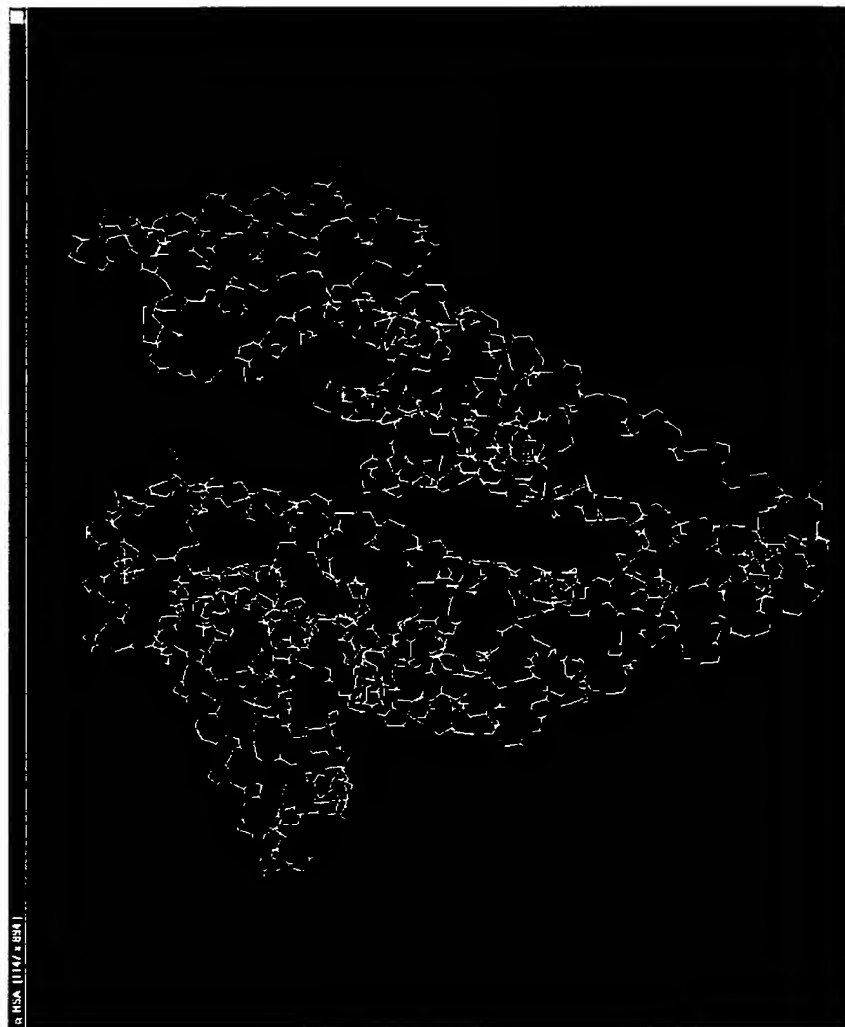


Figure 13: Tertiary Structure of HA

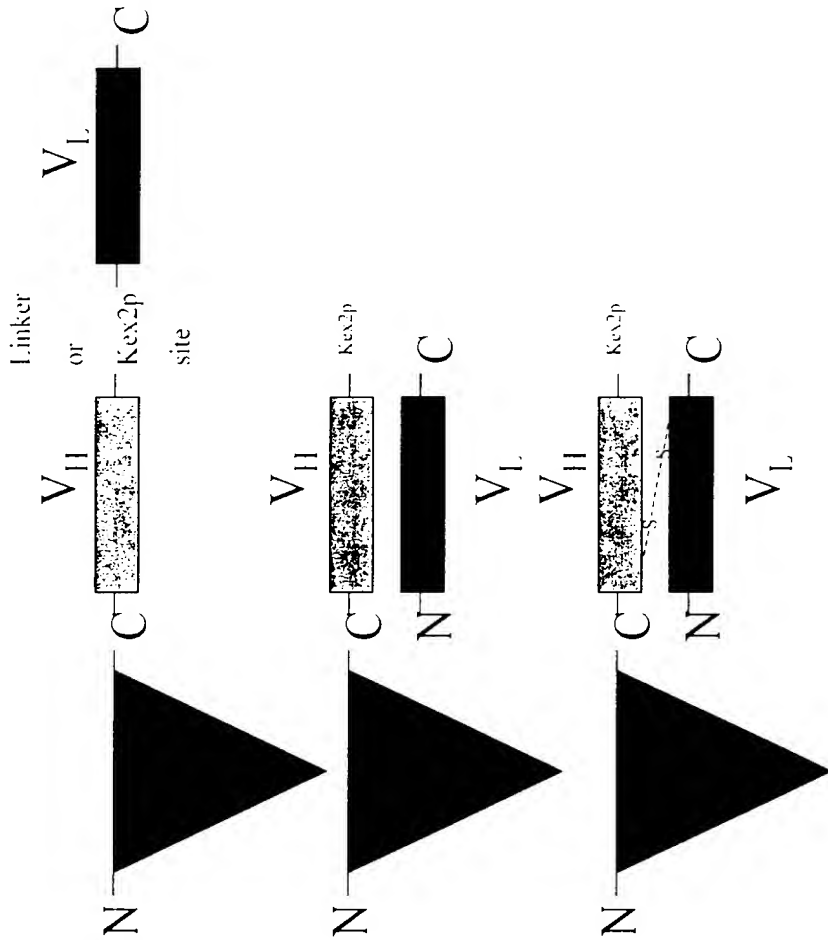


Figure 14: Schematic Diagram of Possible ScFv Fusions
 (Example is of a C-terminal fusion to HA)

1 GAT GCA CAC AAG ACT GAG GTT GCT CAT CGG TTT AAA GAT TTG GGA GAA GAA AAT TTC AAA 60
 10 A H K S E V A H R F E D L G E E N F K 20

 61 GAC TTG GTG TTG ATT GCC TTT OCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120
 21 A L V L : A F A Q Y L Q Q C P F E D H V 40

 121 AAA TTA GTG AAT GAG GTA ACT GAA TTT GCA AAA ACA TGT GTT GCT GAT GAG TCA GGT GAA 160
 41 K L V N E V T E F A E T C V A D E S A E 60

 181 AAT TGT GAC AAA TGA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 210
 61 H C D E S L H T L F G D K L C T V A T L 80

 241 CTT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CTT GAG AGA AAT GAA 300
 81 K E T Y G E M A D C C A K Q E P E R H E 100

 301 TGC TTC TTG CAA CAC AAA GAT GAC AAC CCA AAC CTC CCC CCA TTG GTG AGA CCA GAG GTT 360
 101 C F L Q H K D D N P N L P R L V R P E V 120

 361 GAT GTG ATG TGC AAT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420
 121 D V M C T A F H D N E E T F L K F Y L Y 140

 421 GAA ATT GTC AGA AGA GAT CCT TAC TTT TAT GGC CCG GAA CTC CTT TTC TTT GCT AAA AGG 480
 141 E I A R P H P Y F Y A P E L L F F A K R 160

Figure 15A

481 TAT AAA GCT GAT TTT ACA GAA TGT TGC CAA OCT OCT GAT AAA OCT GAT TGC CTG TTG CCA 540
 161 Y K A A F T E C A A D K A A C L L P 180

 541 AAG CTC GAT GAA CTT CCG GAT GAA GGG AAG OCT TCG TCT GCC AAA CAG AGA CTC AAA TGT 600
 181 K L D E L R D E G K A S S A E Q R L K C 200

 601 GGC AGT CTC GAA AAA TTT CGA GAA AGA OCT TTC AAA GCA TGC GCA GTG GCT CGC CTG ACC 660
 201 A S L Q E F G E R A F K A W A V A R L S 220

 661 CAG AAA TTT CCG AAA GAT GAG TTT GCA GAA GTT TTC AAG TTA GTG ACA GAT CTT ACC AAA 720
 221 Q R F P F A E F A E V S F L Y I D L T K 240

 721 CTC CAC AAG GAA TGA TGC GAT GAA GAT CTG GAT TGT GAA TGT GCT GAT GAC AAG GAG GAC CTT 780
 241 V H T E C C H G D L L E C A D D R A D L 260

 781 GGC AAG TAT ATC TGT GAA AAT CAG GAT TCG ATC TCC AGT AAA CTG AAG GAA TGC TGT GAA 840
 261 A K Y I C E N Q D S I S S K L E E C E 280

 841 AAA CTT CTG TTT GAA AAG TGC CAC TCC ATT OCC GAA CTG GAA AAT GAT GAG ATG CTT GCT 900
 281 E P L L E F S H C I A E V E N D E M P A 300

 901 GAC TTG CTT TTA TTA GCT GAT TTT GAT GAA AGT AAG GAT GTT TGC AAA AAG TAT GCT 960
 301 D L P S L A A D F V E S K D V C K N Y A 320

Figure 15B

961 GAG GCA AAG GAT GTC TTC CTG GGC ATG TTT TTG TAT GAA TAT GCA AGA AGG CAT CCT GAT 1020
 321 E A F D V F L G M F L Y E Y A F R H P D 340

 1021 TAC TCT GTC CTG CTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAG AAG TCC 1080
 341 Y S V V L L L R L A K T Y E T T L E K C 360

 1081 TCT GTC GCT GCA GAT CTT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CTT 1140
 361 C A A A D P H E C Y A K V F D E F K P L 380

 1141 CTG GAA GAG CTT CAG AAT TTA ATC AAA CAA AAC TGT GAG CTT TTT GAG CAG CTT GGA GAG 1200
 381 V E E P Q H L I K Q N C E L F E Q L G E 400

 1201 TAC AAA TTC CAG AAT GAG CTA TTA GTT CTT TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT 1260
 401 Y F F Q H A L L V R Y T K F V P Q V S T 420

 1261 CCA ACT CTT GCA GAG CTG TCA AGA AAC CTA GGA AAA GTG GGC AGC AAA TGT TGT AAA CAT 1320
 421 P T L V E V S R N L G K V G S K C C K H 440

 1321 CTT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CTG AAC CAG TTA 1380
 441 P E A K R M P C A E D Y L S V V L H Q L 460

 1381 TCT GTG TTG CAT GAG AAA ACG CCA GTA AGT GAC AGA GTG ACA AAA TGC TGC ACA GAG TCC 1440
 461 C V L H E K T P V S D R V T K C C T E S 480

Figure 15C

1441 TGG GTG AAC AAG CAA CCA TGC TTT TCA GCT CTG GAA GTC GAT GAA ACA TAC GTT CCC AAA 1500
 481 L V H R P P C F S A L E V D E T Y V P K 506

1501 GAG TTT AAT GCT GAA ACA ACT TTC ACC TTC CAT GCA GAT ATA TGC ACA GTT TCT GAG AAG GAG 1560
 501 E F H A E T F T F H A D I C T L S E K E 520

1561 AGA CAA ATC AAG AAA CAA ACT GCA CTT GTT GAG CTT GTG AAA CAC AAG CCC AAG GCA ACA 1620
 521 R Q I K K Q T A L V E L V K K H K P K A T 540

1621 AAA GAG CAA CTG AAA SCT GTT ATG GAT TTC GCA GCT TTT GTA GAG AAG TAC TGC AAG 1680
 541 E E Q L E A V M D D F A A F V E K C C F 560

1681 GCT GAC GAT AAG GAG ACC TGC TTT GGC GAG GAG GGT AAA AAA GTT GTT GCT GCA AST CAA 1740
 561 A D D K E T C F A E E G K K L V A A S Q 580

1741 GCT GCC TTA GAC TTA TAA CTA CAT TTA AAA GCA TCT CAG 1782
 581 A A L G L 585

Figure 15D